THERAPEUTIC USE OF ALPHA-2-DELTA LIGANDS

This invention relates to the use of an alpha-2-delta ligand in the treatment of chronic obstructive pulmonary disease (COPD) and related diseases and treatment of chronic cough, which may be unrelated to COPD.

An alpha-2-delta receptor ligand is any molecule which binds to any sub-type of the human calcium channel alpha-2-delta sub-unit. The calcium channel alpha-2-delta sub-unit comprises a number of receptor sub-types which have been described in the literature: e.g. N. S. Gee, J. P. Brown, V. U. Dissanayake, J. Offord, R. Thurlow, and G. N. Woodruff, *J-Biol-Chem* 271 (10):5768-76, 1996, (type 1); Gong, J. Hang, W. Kohler, Z. Li, and T-Z. Su, *J.Membr.Biol.* 184 (1):35-43, 2001, (types 2 and 3); E. Marais, N. Klugbauer, and F. Hofmann, *Mol.Pharmacol.* 59 (5):1243-1248, 2001. (types 2 and 3); and N. Qin, S. Yagel, M. L. Momplaisir, E. E. Codd, and M. R. D'Andrea. *Mol.Pharmacol.* 62 (3):485-496, 2002, (type 4). They may also be referred to as GABA analogs.

Alpha-2-delta ligands have been described for a number of indications. The best known alpha-2-delta ligand, gabapentin (Neurontin®), 1-(aminomethyl)-cyclohexylacetic acid, was first described in the patent literature in the patent family comprising US4024175. The compound is approved for the treatment of epilepsy and neuropathic pain.

A second alpha-2-delta ligand, pregabalin, (S)-(+)-4-amino-3-(2-methylpropyl)butanoic acid, is described in European patent application publication number EP641330 as an anti-convulsant treatment useful in the treatment of epilepsy and in EP0934061 for the treatment of pain.

Further, the International Patent application arising from GB Patent Application No. GB0225379.7, unpublished at the filing date of the present invention, describes the use of novel and known alpha-2-delta ligand compounds of the formula (I) for use in therapy, particularly pain:

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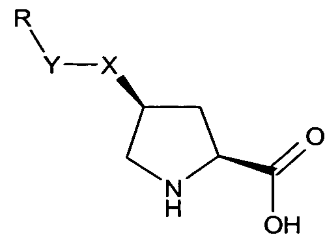


(I)

wherein

either X is O, S, NH or CH_2 and Y is CH_2 or a direct bond, or Y is O, S or NH and X is CH_2; and

R is a 3-12 membered cycloalkyl, 4-12 membered heterocycloalkyl, aryl or heteroaryl, where any ring may be optionally substituted with one or more substituents independently selected from

halogen, hydroxy, cyano, nitro, amino, hydroxycarbonyl,

C_1-C_6 alkyl, C_1-C_6 alkenyl, C_1-C_6 alkynyl,

C_1-C_6 alkoxy, hydroxyC_1-C_6 alkyl, C_1-C_6 alkoxyC_1-C_6 alkyl, perfluoro C_1-C_6 alkyl, perfluoroC_1-C_6 alkoxy,

C_1-C_6 alkylamino, di- C_1-C_6 alkylamino, aminoC_1-C_6 alkyl, C_1-C_6 alkylaminoC_1-C_6 alkyl, di-C_1-C_6 alkylaminoC_1-C_6 alkyl,

C_1-C_6acyl, C_1-C_6acyloxy, C_1-C_6acyloxyC_1-C_6 alkyl, C_1-C_6 acylamino,

C_1-C_6 alkylthio, C_1-C_6 alkylthiocarbonyl, C_1-C_6 alkylthioxo, C_1-C_6 alkoxycarbonyl,

C_1-C_6 alkylsulfonyl, C_1-C_6 alkylsulfonylamino,

aminosulfonyl, C_1-C_6 alkylaminosulfonyl, di-C_1-C_6 alkylaminosulfonyl,

3-8 membered cycloalkyl, 4-8 membered heterocycloalkyl, phenyl and monocyclic heteroaryl;

or a pharmaceutically acceptable salt, solvate or pro-drug thereof. Particular compounds disclosed include: (2S, 4S)-4-(3-Chloro-phenoxy)-pyrrolidine-2-carboxylic acid; (2*S*,4*S*)-4-(3-Fluoro-benzyl)-pyrrolidine-2-carboxylic acid; (2*S*,4*S*)-4-(2,3-Difluoro-benzyl)-pyrrolidine-2-carboxylic acid; (2*S*,4*S*)-4-(2,5-Difluoro-benzyl)-pyrrolidine-2-carboxylic acid; (2*S*,4*S*)-4-Cyclohexylmethyl-pyrrolidine-2-carboxylic acid; (2*S*,4*S*)-4-(3-Fluoro-phenoxymethyl)-pyrrolidine-2-carboxylic acid; (2*S*,4*S*)-4-(3,6-Difluoro-phenoxymethyl)-pyrrolidine-2-carboxylic acid; (2*S*,4*S*)-4-(2,3-Difluoro-phenoxymethyl)-pyrrolidine-2-carboxylic acid; and (2*S*,4*S*)-4-(3-Methoxy- phenoxymethyl)-pyrrolidine-2-carboxylic acid; or a pharmaceutically acceptable salt, solvate or pro-drug thereof.

COPD is a disease state characterized by airflow limitation, that is not fully reversible. The airflow limitation is usually both progressive and associated with an abnormal inflammatory response of the lungs to noxious particles or gases. A diagnosis of COPD should be considered in any patient who has symptoms of cough, sputum production, or dyspnea, and/or a history of exposure to risk factors for the disease. The diagnosis is confirmed by spirometry. Poorly reversible airflow limitation associated with bronchiectasis, cystic fibrosis, tuberculosis, or asthma is not included except insofar as these conditions overlap with COPD. Pathological changes characteristic of COPD are found in the central airways, lung parenchyma, and pulmonary vasculature In the airways, inflammatory cells infiltrate the epithelium. Enlarged mucus-secreting glands and an increase in the number of goblet cells are associated with mucus hypersecretion. Destruction of the lung parenchyma in patients with COPD typically occurs as centrilobular emphysema. This involves dilatation and destruction of the respiratory bronchioles. Pathological changes in the lungs lead to corresponding physiological changes characteristic of the disease, including mucus hypersecretion, ciliary dysfunction, airflow limitation, pulmonary inflation, gas exchange abnormalities, pulmonary hypertension, and cor pulmonale.

Mucus hypersecretion and ciliary dysfunction lead to chronic cough and sputum production. These symptoms can be present for many years before other symptoms or physiologic abnormalities develop.

European Patent Application Publication Number EP1192944 describes the use of pregabalin and related compounds in the treatment of asthma. International Patent Application Publication Number WO00/66096 describes anti-convulsant agents, including gabapentin, for use in the treatment of bronchial conditions. WO00/67742 describes the modulation of substance P by GABA analogs and methods of treating diseases implicated by such modulation, such as respiratory diseases including chronic obstructive airways disease, chronic bronchitis and asthma. Pain, Vol. 105, Issues 1-2, September 2003, pp 133-141, reports that pregabalin and gabapentin reduce release of substance P and CGRP from rat spinal tissues only after inflammation or activation of protein kinase C. Tachykinin receptor antagonists have been postulated for the treatment of respiratory diseases in J. Auton. Pharmacol., 1993; 13,23-93.

It has now been found that alpha-2-delta ligands act by a hitherto unknown mechanism of action at a prejunctional site of action on the cholinergic nerve terminal and are, thus, useful in the treatment, particularly the oral treatment, of COPD and related diseases.

Accordingly, the invention provides, as a first aspect, use of an alpha-2-delta ligand, or a pharmaceutically acceptable salt or solvate thereof, in the manufacture of a medicament for the treatment of COPD and any disease, disorder or condition associated with a diagnosis of COPD.

As a suitable or alternative feature of the present invention, the COPD is COPD that includes chronic bronchitis, pulmonary emphysema or dyspnea associated therewith, or COPD that is characterized by irreversible, progressive airways obstruction, adult respiratory distress syndrome (ARDS) and exacerbation of airways hyper-reactivity consequent to other drug therapy. As a preferred medicament, an oral composition is preferred.

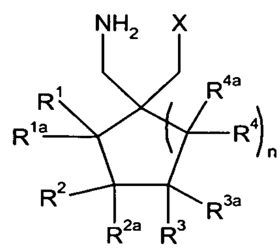
As an alternative or further aspect, the invention provides a method for the curative, prophylactic or palliative treatment of a patient suffering from and any disease, disorder or condition associated with a diagnosis of COPD, comprising administration of an effective amount of an alpha-2-delta ligand, or a pharmaceutically acceptable salt or solvate thereof. As a preferred method, the COPD is COPD that includes chronic bronchitis, pulmonary emphysema or dyspnea associated therewith, or COPD that is characterized by irreversible, progressive airways obstruction, adult respiratory distress syndrome (ARDS) and exacerbation of airways hyper-reactivity consequent to other drug therapy. As a preferred medicament, an oral composition is preferred.

It has further been found that alpha-2-delta ligands are useful in the treatment of chronic cough, which is not necessarily related to COPD, with a likely mechanism via the CNS.

Thus, as an alternative or further aspect of the present invention, there is presented the use of an alpha-2-delta ligand, or a pharmaceutically acceptable salt or solvate thereof, in the manufacture of a medicament for the treatment of chronic cough.

As an alternative aspect, the invention provides a method for the curative, prophylactic or palliative treatment of a patient suffering from chronic cough, comprising administration of an effective amount of an alpha-2-delta ligand, or a pharmaceutically acceptable salt or solvate thereof.

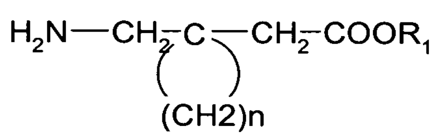
Useful cyclic alpha-2-delta ligands of the present invention are illustrated by the following formula (I):



(I)

wherein X is a carboxylic acid or carboxylic acid bioisostere;
n is 0, 1 or 2; and
R^1, R^{1a}, R^2, R^{2a}, R^3, R^{3a}, R^4 and R^{4a} are independently selected from H and C_1-C_6 alkyl, or R^1 and R^2 or R^2 and R^3 are taken together to form a C_3-C_7 cycloalkyl ring, which is optionally substituted with one or two substituents selected from C_1-C_6 alkyl, or a pharmaceutically acceptable salt thereof.

As an additional aspect of the present invention, there is provided use of a compound of formula (I), as defined above, excluding a compound of formula (Ia)



(Ia)

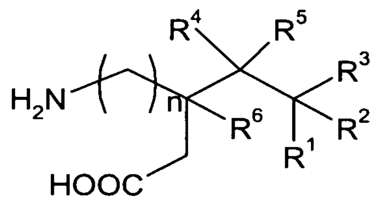
wherein R_1 is hydrogen or a lower alkyl group of up to 8 carbon atoms; n is an integer of from 4 to 6; and the cyclic ring is optionally substituted, and the individual diastereomeric or enantiomeric isomers thereof; and pharmaceutically acceptable salts thereof, particularly gabapentin.

In formula (I), suitably, R^1, R^{1a}, R^{2a}, R^{3a}, R^4 and R^{4a} are H and R^2 and R^3 are independently selected from H and methyl, or R^{1a}, R^{2a}, R^{3a} and R^{4a} are H and R^1 and R^2 or R^2 and R^3 are taken

together to form a C_3-C_7 cycloalkyl ring, which is optionally substituted with one or two methyl substituents. A suitable carboxylic acid bioisostere is selected from tetrazolyl and oxadiazolonyl. X is preferably a carboxylic acid.

In formula (I), preferably, R^1, R^{1a}, R^{2a}, R^{3a}, R^4 and R^{4a} are H and R^2 and R^3 are independently selected from H and methyl, or R^{1a}, R^{2a}, R^{3a} and R^{4a} are H and R^1 and R^2 or R^2 and R^3 are taken together to form a C_4-C_5 cycloalkyl ring, or, when n is 0, R^1, R^{1a}, R^{2a}, R^{3a}, R^4 and R^{4a} are H and R^2 and R^3 form a cyclopentyl ring, or, when n is 1, R^1, R^{1a}, R^{2a}, R^{3a}, R^4 and R^{4a} are H and R^2 and R^3 are both methyl or R^1, R^{1a}, R^{2a}, R^{3a}, R^4 and R^{4a} are H and R^2 and R^3 form a cyclobutyl ring, or, when n is 2, R^1, R^{1a}, R^2, R^{2a}, R^3, R^{3a}, R^4 and R^{4a} are H, or, n is 0, R^1, R^{1a}, R^{2a}, R^{3a}, R^4 and R^{4a} are H and R^2 and R^3 form a cyclopentyl ring.

Useful acyclic alpha-2-delta ligands of the present invention are illustrated by the following formula (II):

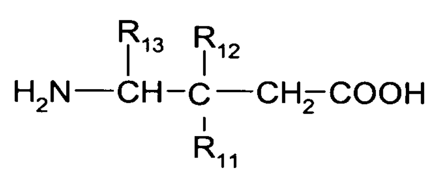


(II)

wherein:

n is 0 or 1, R^1 is hydrogen or $(C_1$-$C_6)$alkyl; R^2 is hydrogen or $(C_1$-$C_6)$alkyl; R^3 is hydrogen or $(C_1$-$C_6)$alkyl; R^4 is hydrogen or $(C_1$-$C_6)$alkyl; R^5 is hydrogen or $(C_1$-$C_6)$alkyl and R^6 is hydrogen or $(C_1$-$C_6)$alkyl, or a pharmaceutically acceptable salt thereof.

As an additional aspect of the present invention, there is provided use of a compound of formula (I), as defined above, excluding a compound of formula (IIa)

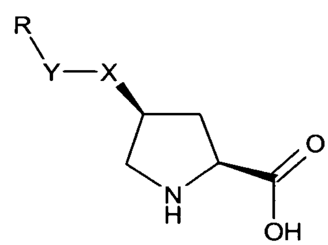


(IIA)

wherein R_{11} is a straight or branched alkyl of from 1 to 6 carbons, phenyl, or cycloalkyl having from 3 to 6 carbon atoms; R_{12} is hydrogen or methyl; and R_{13} is hydrogen methyl or carboxyl; and the individual diastereomeric or enantiomeric isomers thereof; and pharmaceutically acceptable salts thereof, particularly pregabalin.

According to formula (II), suitably R^1 is C_1-C_6 alkyl, R^2 is methyl, $R^3 - R^6$ are hydrogen and n is 0 or 1. More suitably R^1 is methyl, ethyl, n-propyl or n-butyl, R^2 is methyl, $R^3 - R^6$ are hydrogen and n is 0 or 1. When R^2 is methyl, $R^3 - R^6$ are hydrogen and n is 0, R^1 is suitably ethyl, n-propyl or n-butyl. When R^2 is methyl, $R^3 - R^6$ are hydrogen and n is 1, R^1 is suitably methyl or n-propyl. Compounds of formula (II) are suitably in the 3S,5R configuration.

Further useful cyclic alpha-2-delta ligands of the present invention are illustrated by the following formula (III):



(III)

wherein

either X is O, S, NH or CH_2 and Y is CH_2 or a direct bond, or Y is O, S or NH and X is CH_2; and

R is a 3-12 membered cycloalkyl, 4-12 membered heterocycloalkyl, aryl or heteroaryl, where any ring may be optionally substituted with one or more substituents independently selected from

halogen, hydroxy, cyano, nitro, amino, hydroxycarbonyl,

C_1-C_6 alkyl, C_1-C_6 alkenyl, C_1-C_6 alkynyl,

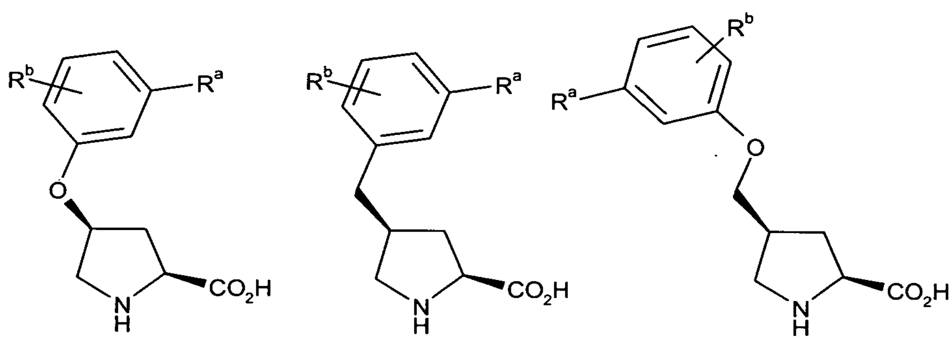
C_1-C_6 alkoxy, hydroxyC_1-C_6 alkyl, C_1-C_6 alkoxyC_1-C_6 alkyl, perfluoro C_1-C_6 alkyl, perfluoroC_1-C_6 alkoxy,

C_1-C_6 alkylamino, di- C_1-C_6 alkylamino, aminoC_1-C_6 alkyl, C_1-C_6 alkylaminoC_1-C_6 alkyl, di-C_1-C_6 alkylaminoC_1-C_6 alkyl,

C_1-C_6acyl, C_1-C_6acyloxy, C_1-C_6acyloxyC_1-C_6 alkyl, C_1-C_6 acylamino,

C_1-C_6 alkylthio, C_1-C_6 alkylthiocarbonyl, C_1-C_6 alkylthioxo, C_1-C_6 alkoxycarbonyl, C_1-C_6 alkylsulfonyl, C_1-C_6 alkylsulfonylamino, aminosulfonyl, C_1-C_6 alkylaminosulfonyl, di-C_1-C_6 alkylaminosulfonyl, 3-8 membered cycloalkyl, 4-8 membered heterocycloalkyl, phenyl and monocyclic heteroaryl; or a pharmaceutically acceptable salt, solvate or pro-drug thereof.

According to formula (III), a suitable sub-group of compounds for the present use invention may be represented by formulae (IIIa), (IIIb) and (IIIc):



(IIIa) (IIIb) (IIIc)

wherein R^a and R^b are independently selected from hydrogen, halogen, hydroxy, (C_1-C_6)alkoxy cyano, nitro, amino, hydroxycarbonyl, C_1-C_6 alkyl, C_1-C_6 alkenyl, C_1-C_6 alkynyl, C_1-C_6 alkoxy, hydroxyC_1-C_6 alkyl, C_1-C_6 alkoxyC_1-C_6 alkyl, perfluoro C_1-C_6 alkyl, perfluoroC_1-C_6 alkoxy, C_1-C_6 alkylamino, di- C_1-C_6 alkylamino, aminoC_1-C_6 alkyl, C_1-C_6 alkylaminoC_1-C_6 alkyl, di-C_1-C_6 alkylaminoC_1-C_6 alkyl, C_1-C_6acyl, C_1-C_6acyloxy, C_1-C_6acyloxyC_1-C_6 alkyl, C_1-C_6 acylamino, C_1-C_6 alkylthio, C_1-C_6 alkylthiocarbonyl, C_1-C_6 alkylthioxo, C_1-C_6 alkoxycarbonyl, C_1-C_6 alkylsulfonyl, C_1-C_6 alkylsulfonylamino, aminosulfonyl, C_1-C_6 alkylaminosulfonyl, di-C_1-C_6 alkylaminosulfonyl, 3-8 membered cycloalkyl, 4-8 membered heterocycloalkyl, phenyl and monocyclic heteroaryl; or a pharmaceutically acceptable salt or solvate thereof.

According to formula (III), particular compounds for use in the present invention include: (2S, 4S)-4-(3-Chloro-phenoxy)-pyrrolidine-2-carboxylic acid; (2*S*,4*S*)-4-(3-Fluoro-benzyl)-pyrrolidine-2-carboxylic acid; (2*S*,4*S*)-4-(2,3-Difluoro-benzyl)-pyrrolidine-2-carboxylic acid; (2*S*,4*S*)-4-(2,5-Difluoro-benzyl)-pyrrolidine-2-carboxylic acid; (2*S*,4*S*)-4-Cyclohexylmethyl-pyrrolidine-2-carboxylic acid; (2*S*,4*S*)-4-(3-Fluoro-phenoxymethyl)-pyrrolidine-2-carboxylic acid;

(2*S*,4*S*)-4-(3,6-Difluoro-phenoxymethyl)-pyrrolidine-2-carboxylic acid; (2*S*,4*S*)-4-(2,3-Difluoro-phenoxymethyl)-pyrrolidine-2-carboxylic acid; and (2*S*,4*S*)-4-(3-Methoxy-phenoxymethyl)-pyrrolidine-2-carboxylic acid; or a pharmaceutically acceptable salt or solvate thereof, which may be prepared by methods well known in the art or with reference to methods described herein.

Examples of alpha-2-delta ligands for use with the present invention are those compounds generally or specifically disclosed in US4024175, particularly gabapentin, EP641330, particularly pregabalin, US5563175, WO9733858, WO9733859, WO9931057, WO9931074, WO9729101, WO02085839, particularly [(1R,5R,6S)-6-(Aminomethyl)bicyclo[3.2.0]hept-6-yl]acetic acid, WO9931075, particularly 3-(1-Aminomethyl-cyclohexylmethyl)-4H-[1,2,4]oxadiazol-5-one and C-[1-(1H-Tetrazol-5-ylmethyl)-cycloheptyl]-methylamine, WO9921824, particularly (3S,4S)-(1-Aminomethyl-3,4-dimethyl-cyclopentyl)-acetic acid, WO0190052, WO0128978, particularly (1α,3α,5α)(3-amino-methyl-bicyclo[3.2.0]hept-3-yl)-acetic acid, EP0641330, WO9817627, WO0076958, particularly (3S,5R)-3-aminomethyl-5-methyl-octanoic acid, WO03/082807, particularly (3S,5R)-3-amino-5-methyl-heptanoic acid, (3S,5R)-3-amino-5-methyl-nonanoic acid and (3S,5R)-3-Amino-5-methyl-octanoic acid, the PCT application arising from GB Patent Application No. GB0225379.7, particularly (2S, 4S)-4-(3-Chloro-phenoxy)-pyrrolidine-2-carboxylic acid, (2*S*,4*S*)-4-(3-Fluoro-benzyl)-pyrrolidine-2-carboxylic acid, (2*S*,4*S*)-4-(2,3-Difluoro-benzyl)-pyrrolidine-2-carboxylic acid, and (2*S*,4*S*)-4-(3-fluoro-phenoxymethyl)-pyrrolidine-2-carboxylic acid, EP1178034, EP1201240, WO9931074, WO03000642, WO0222568, WO023087, WO0230881, WO02100392, WO02100347, WO0242414, WO0232736 and WO0228881, or pharmaceutically acceptable salts thereof, all of which are incorporated herein by reference.

Preferred alpha-2-delta ligands of the present invention include: gabapentin, pregabalin, [(1R,5R,6S)-6-(Aminomethyl)bicyclo[3.2.0]hept-6-yl]acetic acid, 3-(1-Aminomethyl-cyclohexylmethyl)-4H-[1,2,4]oxadiazol-5-one, (3S,4S)-(1-Aminomethyl-3,4-dimethyl-cyclopentyl)-acetic acid, (1α,3α,5α)(3-amino-methyl-bicyclo[3.2.0]hept-3-yl)-acetic acid, (3S,5R)-3-Aminomethyl-5-methyl-octanoic acid, (3S,5R)-3-amino-5-methyl-heptanoic acid, (3S,5R)-3-amino-5-methyl-nonanoic acid and (3S,5R)-3-Amino-5-methyl-octanoic acid, (2S, 4S)-4-(3-Chloro-phenoxy)-pyrrolidine-2-carboxylic acid, (2*S*,4*S*)-4-(3-Fluoro-benzyl)-pyrrolidine-2-carboxylic acid, (2*S*,4*S*)-4-(2,3-Difluoro-benzyl)-pyrrolidine-2-carboxylic acid, and (2*S*,4*S*)-4-(3-fluoro-phenoxymethyl)-pyrrolidine-2-carboxylic acid, or pharmaceutically acceptable salts thereof. Particularly preferred alpha-2-delta ligands of the present invention are selected from gabapentin, pregabalin and (1α,3α,5α)(3-amino-methyl-bicyclo[3.2.0]hept-3-yl)-acetic acid, or pharmaceutically acceptable salts thereof. A most preferred compound of the invention is (1α,3α,5α)(3-amino-methyl-bicyclo[3.2.0]hept-3-yl)-acetic acid, or a pharmaceutically acceptable salt thereof.

The suitability of any alpha-2-delta ligand can be readily determined by evaluation of its potency and selectivity using literature methods, followed by evaluation of its toxicity, absorption, metabolism, pharmacokinetics, etc in accordance with standard pharmaceutical practices.

A compound of the present invention in a single dosage form is suitable for administration to any mammalian subject, preferably human. Administration may be once (o.d.), twice (b.i.d.) or three times (t.i.d.) daily, suitably b.i.d. or t.i.d., more suitably b.i.d, most suitably o.d..

The invention may be illustrated with reference to the following the following figures:

Figure 1: Effect of gabapentin on human small bronchi contraction produced by electric field stimulation (EFS).

Figure 2: Absence of effect of gabapentin on human small bronchi contraction induced by acethylcholine

Figure 3: Effect of Compound A on human small bronchi contraction produced by electric field stimulation (EFS).

Figure 4: Absence of effect of Compound A on human small bronchi contraction induced by acethylcholine

Figure 5: Effect of inhaled Compound A and gabapentin on citric acid-induced cough in guinea-pigs

Alpha-2-delta ligands for use in the treatment of COPD are also useful in the treatment of any obstructive or inflammatory airways diseases of whatever type, etiology, or pathogenesis, for example, chronic eosinophilic pneumonia. The compounds are also useful in the treatment of any number of symptoms associated with a diagnosis of COPD, for example any condition selected from the group consisting of :

- asthma of whatever type, etiology, or pathogenesis, in particular asthma that is a member selected from the group consisting of atopic asthma, non-atopic asthma, allergic asthma, atopic bronchial IgE mediated asthma, bronchial asthma, essential asthma, true asthma, intrinsic asthma caused by pathophysiologic disturbances, extrinsic asthma caused by environmental factors, essential asthma of unknown or inapparent cause, non-atopic asthma, bronchitic asthma, emphysematous asthma, exercise-induced asthma, allergen induced asthma, cold air induced asthma, occupational asthma, infective asthma caused by bacterial, fungal, protozoal, or viral infection, non-allergic asthma, incipient asthma and wheezy infant syndrome;
- chronic or acute bronchoconstriction, large airway obstruction, chronic bronchitis, small airways obstruction, and emphysema;
- pneumoconiosis of whatever type, etiology, or pathogenesis, in particular pneumoconiosis that is a member selected from the group consisting of aluminosis or bauxite workers' disease, anthracosis or miners' asthma, asbestosis or steam-fitters' asthma, chalicosis or flint disease, ptilosis caused by inhaling the dust from ostrich feathers, siderosis caused by the inhalation of iron particles, silicosis or grinders' disease, byssinosis or cotton-dust asthma and talc pneumoconiosis;
- bronchitis of whatever type, etiology, or pathogenesis, in particular bronchitis that is a member selected from the group consisting of acute bronchitis, acute laryngotracheal bronchitis, arachidic bronchitis, catarrhal bronchitis, croupus bronchitis, dry bronchitis, infectious asthmatic

bronchitis, productive bronchitis, staphylococcus or streptococcal bronchitis and vesicular bronchitis; and

- bronchiectasis of whatever type, etiology, or pathogenesis, in particular bronchiectasis that is a member selected from the group consisting of cylindric bronchiectasis, sacculated bronchiectasis, fusiform bronchiectasis, capillary bronchiectasis, cystic bronchiectasis, dry bronchiectasis and follicular bronchiectasis.

The compounds for use in the present invention are prepared by methods well known to those skilled in the art or as described herein. Specifically, the patents, patent applications and publications, mentioned hereinabove, each of which is hereby incorporated herein by reference, exemplify compounds which can be used in the present invention.

The compounds for use in the present invention can exist in unsolvated forms as well as solvated forms, including hydrated forms. In general, the solvated forms, including hydrated forms, which may contain isotopic substitutions (e.g. D2O, d6-acetone, d6-DMSO), are equivalent to unsolvated forms and are encompassed within the scope of the present invention.

The compounds of formulas (I), (II), and (III) may contain asymmetric or chiral centers and, therefore, exist in different stereoisomeric forms. It is intended that all stereoisomeric forms of the compounds of formulas (I), (II), and (III), as well as mixtures thereof, including for example racemic mixtures and epimers, form part of the present invention. In addition, the present invention embraces all geometric and positional isomers. For example, if a compound of formula (I) incorporates a double bond, both the *cis*- and *trans*- forms, as well as mixtures thereof, are embraced within the scope of the invention.

Diastereomeric mixtures can be separated into their individual diastereomers on the basis of their physical chemical differences by methods well-known to those of ordinary skill in the art, such as by chromatography and/or fractional crystallization. Enantiomers can be separated by converting the enantiomeric mixture into a diastereomeric mixture by reaction with an appropriate optically active compound (e.g., alcohol), separating the diastereomers and converting (e.g., hydrolyzing) the individual diastereomers to the corresponding pure enantiomers.

A number of the alpha-2-delta ligands of the present use invention are amino acids. Since amino acids are amphoteric, pharmacologically compatible salts can be salts of appropriate non-toxic inorganic or organic acids or bases. Suitable acid addition salts are the acetate, aspartate, benzoate, besylate, bicarbonate/carbonate, bisulphate, camsylate, citrate, edisylate, esylate, fumarate, gluceptate, gluconate, glucuronate, hibenzate, hydrochloride/chloride, hydrobromide/bromide, hydroiodide/iodide, hydrogen phosphate, isethionate, D- and L-lactate, malate, maleate, malonate, mesylate, methylsulphate, 2-napsylate, nicotinate, nitrate, orotate, palmoate, phosphate, saccharate, stearate, succinate sulphate, D- and L-tartrate, and tosylate salts. Suitable base salts are formed from bases which form non-toxic salts and examples are the sodium, potassium, aluminium, calcium, magnesium, zinc, choline, diolamine, olamine, arginine, glycine, tromethamine, benzathine, lysine, meglumine and diethylamine salts. Salts with quaternary ammonium ions can also be prepared with, for example, the tetramethyl-ammonium ion. The compounds of the invention may also be formed as a zwitterion.

A suitable salt for amino acid compounds of the present use invention is the hydrochloride salt. For a review on suitable salts see Stahl and Wermuth, Handbook of Pharmaceutical Salts: Properties, Selection, and Use, Wiley-VCH, Weinheim, Germany (2002).

Also within the scope of the invention are use of clathrates, drug-host inclusion complexes wherein, in contrast to the aforementioned solvates, the drug and host are present in non-stoichiometric amounts. For a review of such complexes, see J Pharm Sci, 64 (8), 1269-1288 by Haleblian (August 1975).

Hereinafter all references to compounds of the use invention include references to salts thereof and to solvates and clathrates of compounds of the invention and salts thereof.

Also included within the present scope of the compounds of the use invention are polymorphs thereof.

Prodrugs of the above compounds of the use invention are included in the scope of the instant invention. The chemically modified drug, or prodrug, should have a different

pharmacokinetic profile to the parent, enabling easier absorption across the mucosal epithelium, better salt formulation and/or solubility, improved systemic stability (for an increase in plasma half-life, for example). These chemical modifications may be

(1) Ester or amide derivatives which may be cleaved by, for example, esterases or lipases. For ester derivatives, the ester is derived from the carboxylic acid moiety of the drug molecule by known means. For amide derivatives, the amide may be derived from the carboxylic acid moiety or the amine moiety of the drug molecule by known means.

(2) Peptides which may be recognized by specific or nonspecific proteinases. A peptide may be coupled to the drug molecule via amide bond formation with the amine or carboxylic acid moiety of the drug molecule by known means.

(3) Derivatives that accumulate at a site of action through membrane selection of a prodrug form or modified prodrug form.

(4) Any combination of 1 to 3.

Aminoacyl-glycolic and -lactic esters are known as prodrugs of amino acids (Wermuth C.G., *Chemistry and Industry*, 1980:433-435). The carbonyl group of the amino acids can be esterified by known means. Prodrugs and soft drugs are known in the art (Palomino E., *Drugs of the Future*, 1990;15(4):361-368). The last two citations are hereby incorporated by reference.

The biological activity of the alpha-2-delta ligands of the use invention may be measured in a radioligand binding assay using [^{3}H]gabapentin and the $\alpha_2\delta$ subunit derived from porcine brain tissue (Gee N.S., Brown J.P., Dissanayake V.U.K., Offord J., Thurlow R., Woodruff G.N., *J. Biol. Chem.*, 1996;271:5879-5776). Results may be expressed in terms of μM or nM $\alpha 2\delta$ binding affinity.

The alpha-2-delta ligands for use in the present invention may be administered separately, simultaneously or sequentially in combination with other therapeutic agents. Suitable optional agents include:

(a) 5-Lipoxygenase (5-LO) inhibitors or 5-lipoxygenase activating protein (FLAP) antagonists,

(b) Leukotriene antagonists (LTRAs) including antagonists of LTB4, LTC4, LTD4, and LTE4,

(c) Histaminic receptor antagonists including H1, H3 and H4 antagonists,

(d) α1- and α2-adrenoceptor agonist vasoconstrictor sympathomimetic agents for decongestant use,

(e) Muscarinic M3 receptor antagonists or anticholinergic agents,

(f) β2-adrenoceptor agonists,

(g) Theophylline,

(h) Sodium cromoglycate,

(i) COX-1 inhibitors (NSAIDs) and COX-2 selective inhibitors,

(j) Oral or inhaled Glucocorticosteroids,

(k) Monoclonal antibodies active against endogenous inflammatory entities,

(l) Anti-tumor necrosis factor (anti-TNF-a) agents,

(m) Adhesion molecule inhibitors including VLA-4 antagonists,

(n) Kinin-B1 - and B2 -receptor antagonists,

(o) Immunosuppressive agents,

(p) Inhibitors of matrix metalloproteases (MMPs),

(q) Tachykinin NK1, NK2 and NK3 receptor antagonists,

(r) Elastase inhibitors,

(s) Adenosine A2a receptor agonists,

(t) Inhibitors of urokinase,

(u) Compounds that act on dopamine receptors, e.g. D2 agonists,

(v) Modulators of the NFkb pathway, e.g. IKK inhibitors,

(w) Agents that can be classed as mucolytics or anti-tussive,

(x) antibiotics,

(y) p38 MAP kinase inhibitors, and

(z) PDE4 inhibitors

The present invention extends to use of an alpha-2-delta ligand, or a pharmaceutically acceptable salt or solvate thereof, in the manufacture of a medicament, in combination with one or more other therapeutic agents, particularly one or more selected from (a)-(z) above, for the treatment of COPD and related diseases or in the treatment of chronic cough.

The compounds for use in the invention can be administered alone but will generally be administered in an admixture with suitable pharmaceutical excipient(s), diluent(s) or carrier(s) selected with regard to the intended route of administration and standard pharmaceutical practice. If appropriate, auxiliaries can be added. Auxiliaries are preservatives, anti-oxidants, flavours or colourants. The compounds for use in the invention may be of immediate-, delayed-, modified-, sustained-, pulsed- or controlled-release type.

The compounds for use in the present invention can be administered, for example but not limited to, the following route: orally, buccally or sublingually in the form of tablets, capsules, multi-and nano-particulates, gels, films (incl. muco-adhesive), powder, ovules, elixirs, lozenges (incl. liquid-filled), chews, solutions, suspensions and sprays. The compounds of the invention may also be administered as osmotic dosage form, or in the form of a high energy dispersion or as coated particles or fast-dissolving, fast -disintegrating dosage form as described in Ashley Publications, 2001 by Liang and Chen. The compounds for use in the invention may be administered as crystalline or amorphous products, freeze dried or spray dried. Suitable formulations of the compounds of the invention may be in hydrophilic or hydrophobic matrix, ion-exchange resin complex, coated or uncoated form and other types as described in US 6,106,864 as desired.

Such pharmaceutical compositions, for example, tablets, may contain excipients such as microcrystalline cellulose, lactose, sodium citrate, calcium carbonate, dibasic calcium phosphate, glycine and starch (preferably corn, potato or tapioca starch), mannitol, disintegrants such as sodium starch glycolate, crosscarmellose sodium and certain complex silicates, and granulation binders such as polyvinylpyrrolidone, hydroxypropylmethylcellulose (HPMC), triglycerides, hydroxypropylcellulose (HPC), bentonite sucrose, sorbitol, gelatin and acacia. Additionally, lubricating agents may be added to solid compositions such as magnesium stearate, stearic acid,

glyceryl behenate, PEG and talc or wetting agents, such as sodium lauryl sulphate. Additionally, polymers such as carbohydrates, phospoholipids and proteins may be included.

Fast dispersing or dissolving dosage fromulations (FDDFs) may contain the following ingredients: aspartame, acesulfame potassium, citric acid, croscarmellose sodium, crospovidone, diascorbic acid, ethyl acrylate, ethyl cellulose, gelatin, hydroxypropylmethyl cellulose, magnesium stearate, mannitol, methyl methacrylate, mint flavouring, polyethylene glycol, fumed silica, silicon dioxide, sodium starch glycolate, sodium stearyl fumarate, sorbitol or xylitol. The terms dispersing or dissolving as used herein to describe FDDFs are dependent upon the solubility of the drug substance used, i.e. where the drug substance is insoluble a fast dispersing dosage form can be prepared and where the drug substance is soluble a fast dissolving dosage form can be prepared.

The solid dosage form, such as tablets are manufactured by a standard process, for example, direct compression or a wet, dry or melt granulation, melt congealing and extrusion process. The tablet cores which may be mono or multi-layer may be coated with appropriate overcoats known in the art.

Solid compositions of a similar type may also be employed as fillers in capsules such as gelatin, starch or HPMC capsules. Preferred excipients in this regard include lactose, starch, a cellulose, milk sugar or high molecular weight polyethylene glycols. Liquid compositions may be employed as fillers in soft or hard capsules such as gelatin capsule. For aqueous and oily suspensions, solutions, syrups and/or elixirs, the compounds of the invention may be combined with various sweetening or flavouring agents, colouring matter or dyes, with emulsifying and/or suspending agents and with diluents such as water, ethanol, propylene glycol, methylcellulose, alginic acid or sodium alginate, glycerin, oils, hydrocolloid agents and combinations thereof. Moreover, formulations containing these compounds and excipients may be presented as a dry product for constitution with water or other suitable vehicles before use.

Liquid form preparations include solutions, suspensions, and emulsions, for example, water or water propylene glycol solutions. For parenteral injection, liquid preparations can be formulated in solution in aqueous polyethylene glycol solution. Aqueous solutions suitable for oral use can be

prepared by dissolving the active component in water and adding suitable colorants, flavors, stabilizing and thickening agents as desired. Aqueous suspensions suitable for oral use can be made by dispersing the finely divided active component in water with viscous material, such as natural or synthetic gums, resins, methylcellulose, sodium carboxymethylcellulose, and other well-known suspending agents.

The compounds for use in the present invention can also be administered by injection, that is, intravenously, intramuscularly, intracutaneously, intraduodenally, or intraperitoneally, intraarterially, intrathecally, intraventricularly, intraurethrally, intrasternally, intracranially, intraspinally or subcutaneously, or they may be administered by infusion, needle-free injectors or implant injection techniques. For such parenteral administration they are best used in the form of a sterile aqueous solution, suspension or emulsion (or system so that can include micelles) which may contain other substances known in the art, for example, enough salts or carbohydrates such as glucose to make the solution isotonic with blood. The aqueous solutions should be suitably buffered (preferably to a pH of from 3 to 9), if necessary. For some forms of parenteral administration they may be used in the form of a sterile non-aqueous system such as fixed oils, including mono- or diglycerides, and fatty acids, including oleic acid. The preparation of suitable parenteral formulations under sterile conditions for example lyophilisation is readily accomplished by standard pharmaceutical techniques well-known to those skilled in the art. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle (e.g. sterile, pyrogen-free water) before use.

Also, the compounds for use in the present invention can be administered intranasally or by inhalation. They are conveniently delivered in the form of a dry powder (either alone, as a mixture, for example a dry blend with lactose, or a mixed component particle, for example with phospholipids) from a dry powder inhaler or an aerosol spray presentation from a pressurised container, pump, spray, atomiser (preferably an atomiser using electrohydrodynamics to produce a fine mist) or nebuliser, with or without the use of a suitable propellant, e.g. dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, a hydrofluoroalkane such as 1,1,1,2-tetrafluoroethane (HFA 134A [trade mark]) or 1,1,1,2,3,3,3-heptafluoropropane (HFA 227EA [trade mark]), carbon dioxide, a further perfluorinated hydrocarbon such as

Perflubron (trade mark) or other suitable gas. In the case of a pressurised aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount. The pressurised container, pump, spray, atomiser or nebuliser may contain a solution or suspension of the active compound, e.g. using a mixture of ethanol (optionally, aqueous ethanol) or a suitable agent for dispersing, solubilising or extending release and the propellant as the solvent, which may additionally contain a lubricant, e.g. sorbitan trioleate. Capsules, blisters and cartridges (made, for example, from gelatin or HPMC) for use in an inhaler or insufflator may be formulated to contain a powder mix of the compound of the invention, a suitable powder base such as lactose or starch and a performance modifier such as l-leucine, mannitol or magnesium stearate.

Prior to use in a dry powder formulation or suspension formulation for inhalation the compounds for use in the invention will be micronised to a size suitable for delivery by inhalation (typically considered as less than 5 microns). Micronisation could be achieved by a range of methods, for example spiral jet milling, fluid bed jet milling, use of supercritical fluid crystallisation or by spray drying.

A suitable solution formulation for use in an atomiser using electrohydrodynamics to produce a fine mist may contain from 1μg to 10mg of the compound for use in the invention per actuation and the actuation volume may vary from 1 to 100μl. A typical formulation may comprise a compound of the invention, propylene glycol, sterile water, ethanol and sodium chloride. Alternative solvents may be used in place of propylene glycol, for example glycerol or polyethylene glycol.

Alternatively, the compounds for use in the invention may be administered topically to the skin, mucosa, dermally or transdermally, for example, in the form of a gel, hydrogel, lotion, solution, cream, ointment, dusting powder, dressing, foam, film, skin patch, wafers, implant, sponges, fibres, bandage, microemulsions and combinations thereof. For such applications, the compounds of the invention can be suspended or dissolved in, for example, a mixture with one or more of the following: mineral oil, liquid petrolatum, white petrolatum, propylene glycol, polyoxyethylene polyoxypropylene compound, emulsifying wax , fixed oils, including synthetic mono- or diglycerides, and fatty acids, including oleic acid, water, sorbitan monostearate, a

polyethylene glycol, liquid paraffin, polysorbate 60, cetyl esters wax, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol, alcohols such as ethanol. Alternatively, penetration enhancers may be used. The following may also be used polymers, carbohydrates, proteins, phospolipids in the form of nanoparticles (such as niosomes or liposomes) or suspended or dissolved. In addition, they may be delivered using iontophoresis, electroporation, phonophoresis and sonophoresis.

Alternatively, the compounds for use in the invention can be administered rectally, for example in the form of a suppository or pessary. They may also be administered by vaginal route. For example, these compositions may be prepared by mixing the drug with a suitable non-irritant excipients, such as cocoa butter, synthetic glyceride esters or polyethylene glycols, which are solid at ordinary temperatures, but liquefy and/or dissolve in the cavity to release the drug.

The compounds for use in the invention may also be used in combination with a cyclodextrin. Cyclodextrins are known to form inclusion and non-inclusion complexes with drug molecules. Formation of a drug-cyclodextrin complex may modify the solubility, dissolution rate, taste-masking, bioavailability and/or stability property of a drug molecule. Drug-cyclodextrin complexes are generally useful for most dosage forms and administration routes. As an alternative to direct complexation with the drug the cyclodextrin may be used as an auxiliary additive, e.g. as a carrier, diluent or solubiliser. Alpha-, beta- and gamma-cyclodextrins are most commonly used and suitable examples are described in WO-A-91/11172, WO-A-94/02518 and WO-A-98/55148.

The term 'administered' includes delivery by viral or non-viral techniques. Viral delivery mechanisms include but are not limited to adenoviral vectors, adeno- associated viral (AAV) vectors, herpes viral vectors, retroviral vectors, lentiviral vectors, and baculoviral vectors. Non-viral delivery mechanisms include lipid mediated transfection, lipsomes, immunoliposomes, lipofectin, cationic facial amphiphiles (CFAs) and combinations thereof. The routes for such delivery mechanisms include but are not limited to mucosal, nasal, oral, parenteral, gastrointestinal, topical or sublingual routes.

Thus, as a further aspect of the present invention, there is provided the use of a pharmaceutical composition comprising an alpha-2-delta ligand, or a pharmaceutically acceptable

salt or solvate thereof, and a suitable excipient, diluent or carrier, in the manufacture of a medicament for the treatment of COPD and any disease, disorder or condition associated with a diagnosis of COPD, or in the treatment of chronic cough.

For non-human animal administration, the term 'pharmaceutical' as used herein may be replaced by 'veterinary.'

The pharmaceutical preparation is preferably in unit dosage form. In such form the preparation is subdivided into unit doses containing appropriate quantities of the active component. The unit dosage form can be a packaged preparation, the package containing discrete quantities of preparation, such as packeted tablets, capsules, and powders in vials or ampoules. Also, the unit dosage form can be a capsules, tablet, cachet, or lozenge itself, or it can be the appropriate number of any of these in packaged form. The quantity of active component in a unit dose preparation may be varied or adjusted from 0.1 mg to 1 g according to the particular application and the potency of the active components. In medical use the drug may be administered three times daily as, for example, capsules of 100 or 300 mg. In therapeutic use, the compounds utilized in the pharmaceutical method of this invention are administered at the initial dosage of about 0.01 mg to about 100 mg/kg daily. A daily dose range of about 0.01 mg to about 100 mg/kg is preferred. The dosages, however, may be varied depending upon the requirements of the patient, the severity of the condition being treated, and the compounds being employed. Determination of the proper dosage for a particular situation is within the skill of the art. Generally, treatment is initiated with smaller dosages which are less than the optimum dose of the compounds. Thereafter, the dosage is increased by small increments until the optimum effect under the circumstances is reached. For convenience, the total daily dosage may be divided and administered in portions during the day, if desired.

For veterinary use, a compound for use according to the present invention or veterinarily acceptable salts or solvates thereof, is administered as a suitably acceptable formulation in accordance with normal veterinary practice and the veterinary surgeon will determine the dosing regimen and route of administration which will be most appropriate for a particular animal.

Biology Examples

Study 1 – Effects of Gabapentin and $(1\alpha,3\alpha,5\alpha)$(3-Amino-methyl-bicyclo[3.2.0]hept-3-yl)-acetic acid on Human Bronchi

Materials and Methods

Human Tissues

The human samples were taken from 10 different patients who were undergoing lung resection for a solitary peripheral carcinoma. Nine subjects had a history of cigarette smoking. The bronchial rings (from 3 to 11 from each sample) were taken from the lobar or segmental bronchus of the lobe obtained at surgery, away from the tumor site.

All experiments complied with the national guidelines and were approved by the regional ethics committee.

Organ Bath Studies

Bronchial rings (2-4 mm in diameter) were mounted in 5-ml organ baths containing a modified Krebs solution (118 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 0.5 mM $MgCl_2$, 25 mM $NaHCO_3$,1 mM $NaHPO_4$, and 11.1 mM glucose), maintained at 37°C, and oxygenated with a mixture of 95% O_2 and 5% CO_2. Tissues were fixed to the base of the organ bath and connected to an isometric force transducer. An optimal tension of 2.5 g was applied. During the initial stabilization period (90 min) tissues were washed six times. A challenge with acetyl choline (Ach) (1 mM) was performed and after washing, the tissue was allowed to equilibrate for an additional 90 min. To prevent peptide degradation phosphoramidon (1 μM) was added to the bath. Viability of the tissues was tested with the response to ACh (1 mM). A cumulative concentration-response curve was constructed by applying increasing concentrations of ACh (100 nM – 10 mM) as soon as a plateau was reached with the previous concentration. The effect of pretreatment with gabapentin (0.1 mM and 1 mM, 15 min before the stimulus), $(1\alpha,3\alpha,5\alpha)$(3-amino-methyl-bicyclo[3.2.0]hept-3-yl)-acetic acid (hereinafter referred to as Compound A) (0.1 mM and 1 mM, 15 min before the stimulus) or their respective vehicles was also studied.

In a separate set of experiments, human bronchi were electrically stimulated (30 Hz, 0.5 ms pulse width, duration 10 sec, 70V). A second contractile responses evoked by EFS was obtained at 30 min interval in the presence of gabapentin (0.1 mM and 1 mM), Compound A (0.1 mM and 1 mM) or their vehicle (saline).

Experiments in human bronchi were performed in the presence of L-NAME (100 μM) to block the production of nitric oxide, indomethacin (5 μM) to block the production of prostanoids and propranolol (1 μM) to block ß-adrenoceptors.

Statistical Analysis

Values were presented as mean ± standard error of the mean (SEM). Comparisons between groups were made by one way analysis of variance (ANOVA) and the Dunnett's test for multiple comparisons. A p value of <0.05 was considered significant.

Results and Discussion

In isolated guinea pig bronchi both gabapentin and Compound A showed a significant ($p <0.05$) inhibitory effect on the contractile response produced by EFS (Fig.1 and 3). It should be underlined that in the human airways the sole component of the contractile response to EFS is cholinergic in nature. Thus, the reduction produced by both gabapentin and Compound A should be ascribed to the ability of the two drugs to inhibit the cholinergic contraction. The additional observation that either gabapentin or Compound A did not produce any significant inhibition of the contraction caused by acetylcholine (Fig. 2 and 4) excluded that the two drugs show any appreciable inhibitory effect on muscarinic receptors involved in the cholinergic contraction of the human airways.

Thus, these findings show that both gabapentin and Compound A do not possess antimuscarinic activity, but rather reduce cholinergic transmission in the human airways through an inhibitory action on postganglionic cholinergic neurons. In conclusion the present data indicate that alpha-2-delta reduce the cholinergic component of the contractile response to EFS in human bronchi, presumably acting at a prejunctional level on postganglionic cholinergic neurons.

Study 2 - Effects of Gabapentin and ($1\alpha,3\alpha,5\alpha$)(3-Amino-methyl-bicyclo[3.2.0]hept-3-yl)-acetic acid on Ctric Acid Induced Cough in Guinea Pigs

Materials and Methods

Animals

Dunkin Hartley guinea pigs (200-250 g) (Charles River, Italy) were acclimatized in cages (24 ± 0.5 °C) for 1 week after delivery, and were allowed free access to water and standard rodent diet (Morini, Italy).

Cough Experimental Model.

After the period of acclimatisation to laboratory conditions, animals were individually placed in a transparent perspex box (20 x 10 x 10 cm, Vetrotecnica, Italy) ventilated with a constant airflow of 400 ml/min. The tussive agents (citric acid 0.25 M) was nebulised *via* a mini-ultrasonic nebuliser (Ugo Basile, Italy). The particle size produced had an aerodynamic mass median diameter of 0.9 μm and the output of the nebuliser was 0.4 ml per min. The appearance of cough was detected by means of a tie-clip microphone (Sony, Japan) and confirmed by the characteristic posture of the animal. The cough sounds were recorded and digitally stored. The number of elicited cough efforts was subsequently counted by a blind observer.

All experiments were carried out at the same time of day. To elicit cough, guinea pigs were exposed to aerosols of citric acid for 10 min. To evaluate the effects of aerosolised gabapentin or Compound A on citric acid-induced cough, guinea pigs inhaled for 10 minutes gabapentin (1 mM) or Compound A (1 mM) prior to exposure to the tussigenic agent.

Statistical analysis

Values were presented as mean ± standard error of the mean (SEM). Comparisons between groups were made by one way analysis of variance (ANOVA) and the Dunnett's test for multiple comparisons. A p value of <0.05 was considered significant.

Results and Discussion

Aerosolized gabapentin or Compound A caused a significant inhibition of the cough response to citric acid inhalation (Figure 5). It should be underlined that a similar inhibitory effect on citric acid induced cough was obtained with maximum doses of TRPV1 antagonists (Lalloo *et al.*, "Capsazepine inhibits cough induced by capsaicin and citric acid but not by hypertonic saline in guinea pigs." *J Appl Physiol* 1995;**79**:1082-7; and Trevisani *et al.*, "Iodo-Resiniferatoxin is a Potent Antitussive Drug in Guinea Pigs." *Thorax*, In Press). It is possible that doses lower than that used in the present experiments would exert an inhibitory effect on citric acid induced cough. It was observed that guinea pigs treated with 1 mM (for 10 min) aerosolized gabapentin or Compound A showed some degree of reduction in motor activity. Although this behavioral effect was not quantified it may suggest that at least part of the antitussive action of both gabapentin and Compound A is mediated by a central mechanism of action. Citric acid induced cough in guinea pigs is considered to be due mainly due to the activation of the capsaicin receptor (TRPV1) of A-delta and C fibres of the upper airways, since TRPV1 antagonists as capsazepine (Lalloo *et al.*, *supra*) or iodo-resiniferatoxin (Trevisani *et al.*, *supra*) inhibited this response. Both citric acid and capsaicin are among the most used stimuli in provocation cough studies both in guinea pigs and man. Thus, the present finding suggests that both gabapentin and Compound A may exert some antitussive effect also in humans.

In conclusion the present data indicate that alpha-2-delta ligands exert a marked antitussive effect on citric acid-induced cough in guinea pigs, presumably by a central mechanism.

Chemistry Examples

Example 1: (2S, 4S)-4-(3-Chloro-phenoxy)-pyrrolidine-2-carboxylic acid

(2S, 4S)-4-(3-Chloro-phenoxy)-pyrrolidine-1,2-dicarboxylic acid 1-tert-butyl ester 2-methyl ester

To a stirred solution of (2S, 4R)-4-hydroxy-pyrrolidine-1,2-dicarboxylic acid 1-tert-butyl ester 2-methyl ester (CAS Reg 74844-91-0) (6.1kg,24.87mol), 3-chlorophenol (3.52kg,27.39mol) & triphenylphosphine (7.18kg,27.37mol) in tert-butyl methyl ether (30.5L) at 0'C was added diisopropylazodicarboxylate (5.53kg,27.35mol) in tert-butyl methyl ether (15L) dropwise. The mixture was stirred overnight at 20'C. The reaction was filtered and the liquors washed with 0.5M sodium hydroxide (aq) (2 x 12.5L) & water (12.2L). The tert-butyl methyl ether solvent was replaced with n-heptane (42.7L) by atmospheric pressure distillation & cooled to crystallise crude product, which was collected by filtration (11.1kg, 125% contaminated with ca 35% reduced diisopropyl dicarboxylate & triphenylphosphine oxide - corrected yield = 86%).

LRMS (Electrospray): m/z 378 (MNa^+).

(2S, 4S)-4-(3-Chloro-phenoxy)-pyrrolidine-1,2-dicarboxylic acid 1-tert-butyl ester

To the products of the preceding preparation (11.1kg, 20.28mol) in THF (26.6L) was added a solution of $LiOH.H_2O$ (4.86kg, 115.4mol) in water (55.5L). The mixture was stirred overnight at 25'C. The THF was removed by distillation & the resultant aqueous solution extracted with dichloromethane (33.3L & 16.7L). The combined dichloromethane layers were extracted with water (33L & 16.7L). The combined aqueous phases were adjusted to pH 3-3.5 with 1M hydrochloric acid(aq) & extracted with dichloromethane (2 x 22.2L). The combined dichloromethane phases were replaced with toluene (33.3L), which was cooled to crystallise the product, which was collected by filtration (6.1kg, 98%).

LRMS (Electrospray): m/z [MNa^+] 364, 340 [M-1] 340.

(2S, 4S)-4-(3-Chloro-phenoxy)-pyrrolidine-2-carboxylic acid

A solution of (2S, 4S)-4-(3-Chloro-phenoxy)-pyrrolidine-1,2-dicarboxylic acid 1-tert-butyl ester (29.25mol) was dissolved in THF (20L) & filtered. To this solution was added 4M HCl in dioxane (30L) & stirred overnight. Tert-Butyl methyl ether (70L) was added to the resultant suspension & the product was collected by filtration (7.06kg, 86.7%).

^{1}H NMR (400 MHz, CD_3OD): δ = 2.65 (m, 2H), 3.60 (dd, 1H), 3.70 (d, 1H), 4.60 (dd, 1H), 5.02 (m, 1H), 6.88 (m, 1H), 6.97 (s, 1H), 7.03 (d, 1H), 7.29 (dd, 1H).

LRMS (Electrospray [MH^+] 242, [M-1] 240.

Microanalysis: Found, C, 46.97; H, 4.70; N, 4.90. $C_{11}H_{12}ClNO_3.HCl.0.1H_2O$ requires C, 47.20; H, 4.75; N, 5.00.

Example 2: (2*S*,4*S*)-4-(3-Fluoro-benzyl)-pyrrolidine-2-carboxylic acid mono hydrochloride salt

4-(3-Fluoro-benzyl)-pyrrolidine-1,2-dicarboxylic acid 1-tert-butyl ester 2-(2-isopropyl-5-methyl-cyclohexyl) ester.

4-(3-Fluoro-benzylidene)-pyrrolidine-1,2-dicarboxylic acid 1-tert-butyl ester 2-(2-isopropyl-5-methyl-cyclohexyl) ester (1.20 g, 2.61 mmol) was dissolved in ethyl acetate:toluene (1:1, 12 ml). The solution was submitted to hydrogenation on platinum oxide (120 mg, 10 % by weight) at 25 °C and 15 psi for 1 hour. The reaction mixture was filtered through arbocel and the filtrate reduced under pressure. The residue was purified by flashmaster chromatography eluting with heptane:ethyl actetate (15:1) to yield the title compound as a colourless oil (1.11 g, 91 %).

LRMS (APCI): m/z [MH-BOC]$^+$ 362.

(2*S*,4*S*)-4-(3-Fluoro-benzyl)-pyrrolidine-2-carboxylic acid mono hydrochloride salt

4-(3-Fluoro-benzyl)-pyrrolidine-1,2-dicarboxylic acid 1-tert-butyl ester 2-(2-isopropyl-5-methyl-cyclohexyl) ester (0.91 g, 1.96 mmol) was dissolved in toluene (2 ml). 6N hydrochloric acid (50ml) was added and stirred at reflux for 18 h. The reaction mixture was cooled to room temperature and extracted with ethyl acetate (3 x 20 ml). The aqueous layer was concentrated by evaporated under reduced pressure to give the title compound (417mg, 81 %) as a white solid. ^{1}H-NMR showed a 7:1 ratio of *cis:trans* diastereoisomers so the product was recrystallised from isopropyl alcohol to give the title compound (170mg, 65%) in a ratio of 14:1 *cis:trans* as determined by NMR.

^{1}H-NMR (400MHz, CD_3OD): (mixture of diastereoisomers 2*S*,4*S*:2*S*,4*R* *(14:1)*): δ = 1.85 (q, 1H), 2.51 (quin, 1H), 2.69-2.85 (m, 3H), 3.07 (t, 1H), 3.41 (dd, 1H), 4.38 and 4.48 (t, 1H), 6.90-7.04 (m, 3H), 7.32 (q, 1H).

LRMS (APCI): m/z [MH]$^+$ 224.

$[\alpha]_D^{25}$ −1.27° (c=9.00 in methanol).

Microanalysis: Found C, 55.56; H, 5.81; N, 5.34%. $C_{12}H_{14}FNO_2.HCl$ requires C, 55.50; H, 5.82; N, 5.39%.

Example 3 (2*S*,4*S*)-4-(2,3-Difluoro-benzyl)-pyrrolidine-2-carboxylic acid mono-hydrochloride salt

4-(3-Fluoro-benzyl)-pyrrolidine-1,2-dicarboxylic acid 1-tert-butyl ester 2-(2-isopropyl-5-methyl-cyclohexyl) ester

was prepared by a method analogous to that of 4-(3-Fluoro-benzyl)-pyrrolidine-1,2-dicarboxylic acid 1-tert-butyl ester 2-(2-isopropyl-5-methyl-cyclohexyl) ester using the appropriate starting alkenic menthol ester;

Microanalysis (mixture of diastereoisomers *cis* (major) and *trans*): Found: C, 67.74; H, 8.30; N, 2.90%. $C_{27}H_{39}F_2NO_4$. requires C, 67.62; H, 8.20; N, 2.92%;

$[\alpha]_D^{25}$ -71.92° (c = 3.26 in methanol)

(2*S*,4*S*)-4-(2,3-Difluoro-benzyl)-pyrrolidine-2-carboxylic acid mono-hydrochloride salt

The title compound was made by the same method as for (2*S*,4*S*)-4-(3-Fluoro-benzyl)-pyrrolidine-2-carboxylic acid mono hydrochloride salt, above, starting from 4-(3-Fluoro-benzyl)-pyrrolidine-1,2-dicarboxylic acid 1-tert-butyl ester 2-(2-isopropyl-5-methyl-cyclohexyl) ester, and purified by re-crystallisation with acetone/ether to give the title compound as a mixture of diastereoisomers (2*S*,4*S*:2*S*,4*R* (12:1)) determined by ^{1}H-NMR (500 mg, 60 %) as a white solid.

^{1}H-NMR (400 MHz, CD_3OD) (mixture of diastereoisomers *cis :trans (12:1)*): δ= 0.80-1.90 (m, 0.92H), 2.12-2.20 (m, 0.08H), 2.28-2.36 (m, 0.08H), 2.49-2.58 (q, 0.92H), 2.66-2.81 (m, 1H), 2.83-2.95 (m, 2H), 3.02-3.13 (t, 1H), 3.46 (dd, 1H), 4.40 (dd, 0.92H), 4.48-4.54 (m, 0.08H), 7.03-7.20 (m, 3H).

LRMS (Electrospray): m/z $[M + H]^+$ 242.

Microanalysis: Found C, 51.42; H, 5.08; N, 5.01%. $C_{12}H_{13}NO_2F_2.HCl$ requires C, 51.90; H, 5.08; N, 5.04%.

Example 4

(2*S*,4*S*)-4-(3-fluoro-phenoxymethyl)-pyrrolidine-2-carboxylic acid

<u>(2*S*,4*S*)-Pyrrolidine-1,2,4-tricarboxylic acid 1,2-di-tert-butyl ester</u>

To a mixture of 4-phenyl-pyrrolidine-1,2-dicarboxylic acid di-tert-butyl ester (CAS Reg. No. 344 286-69-7)[1] (0.78g, 2.24mmol) and sodium periodate (5.77g, 27mmol) stirring at 0°C under a nitrogen atmosphere in ethyl acetate (5.5ml), acetonitrile (5.5ml) and water (8.5ml) was added ruthenium trichloride (10mg, 0.05mmol) and stirred to room temperature over 18 hours. Diethyl ether (20ml) was added and stirred for a further 1hr. 1M hydrochloric acid (5ml) was added and the mixture extracted with ethyl acetate (3 x 30ml). Organic extracts were combined, dried ($MgSO_4$), filtered and evaporated under reduced pressure. The residue was purified by chromatography on silica gel, eluting with 50:50:1 ethyl acetate:heptane:glacial acetic acid to give the title compound as a colourless gum (501mg, 78%)

LRMS (electrospray): [M-1] 314

Ref[1] *J. Org. Chem.*, 2001, 3593-3596

<u>(2*S*,4*S*)-4-(3-fluoro-phenoxymethyl)-pyrrolidine-2-carboxylic acid</u>

4-(3-fluoro-phenoxymethyl)-pyrroline-1,2-dicarboxylic acid di-tert-butyl ester (475mg, 1.2mmol) was dissolved in a solution of anhydrous hydrogen chloride in dioxane (4M, 15ml) and stirred at 50°C under a nitrogen atmosphere for 1 hour. The solvent was removed under reduced pressure and the resulting semi-solid triturated with ethyl acetate to give a white solid which was recrystallised from ethyl acetate/isopropyl alcohol to give the title compound as a mixture of diastereomers (~5:1 2*S*,4*S*:2*S*,4*R*) as a white solid hydrochloride salt (90mg, 35%)

^{1}H-NMR (400MHz, CD_3OD): δ = 2.04-2.09 (m, 0.8H); 2.33-2.47 (m, 0.4H); 2.65-2.75 (m, 0.8H); 2.88-3.00 (m, 1H); 3.33-3.40 (m, 1H); 3.52-3.60 (m, 0.8H); 3.60-3.68 (0.2H); 3.96-4.04 (m, 1H); 4.04-4.12 (m, 1H); 4.42-4.51 (m, 0.8H); 4.40-4.56 (m, 0.2H); 6.65-6.80 (m, 3H), 7.21-7.30 (m, 1H)

LRMS (electrospray): [M+1] 240; [M+23] 262; [M-1] 238.

<u>Pharmaceutical Composition Examples</u>

In the following Examples, the term 'active compound' or 'active ingredient' refers to a suitable alpha-2-delta ligand and/or a pharmaceutically acceptable salt or solvate, for use according to the present invention.

(i) Tablet compositions

The following compositions A and B can be prepared by wet granulation of ingredients (a) to (c) and (a) to (d) with a solution of povidone, followed by addition of the magnesium stearate and compression.

Composition A

		mg/tablet	mg/tablet
(a)	Active ingredient	250	250
(b)	Lactose B.P.	210	26
(c)	Sodium Starch Glycollate	20	12
(d)	Povidone B.P.	15	9
(e)	Magnesium Stearate	5	3
		500	300

Composition B

		mg/tablet	mg/tablet
(a)	Active ingredient	250	250
(b)	Lactose 150	150	-
(c)	Avicel PH 101	60	26
(d)	Sodium Starch Glycollate	20	12
(e)	Povidone B.P.	15	9
(f)	Magnesium Stearate	5	3
		500	300

Composition C

mg/tablet

Active ingredient	100
Lactose	200
Starch	50
Povidone	5
Magnesium Stearate	4
	359

The following compositions D and E can be prepared by direct compression of the admixed ingredients. The lactose used in formulation E is of the direct compression type.

Composition D

	mg/tablet
Active ingredient	250
Magnesium Stearate	4
Pregelatinised Starch NF15	146
	400

Composition E

	mg/tablet
Active ingredient	250
Magnesium Stearate	5
Lactose	145
Avicel	100
	500

Composition F (Controlled release composition)

		mg/tablet
(a)	Active ingredient	500
(b)	Hydroxypropylmethylcellulose (Methocel K4M Premium)	112

(c)	Lactose B.P.	53
(d)	Povidone B.P.C.	28
(e)	Magnesium Stearate	7
		700

The composition can be prepared by wet granulation of ingredients (a) to (c) with a solution of povidone, followed by addition of the magnesium stearate and compression.

Composition G (Enteric-coated tablet)

Enteric-coated tablets of Composition C can be prepared by coating the tablets with 25mg/tablet of an enteric polymer such as cellulose acetate phthalate, polyvinylacetate phthalate, hydroxypropylmethyl-cellulose phthalate, or anionic polymers of methacrylic acid and methacrylic acid methyl ester (Eudragit L). Except for Eudragit L, these polymers should also include 10% (by weight of the quantity of polymer used) of a plasticizer to prevent membrane cracking during application or on storage. Suitable plasticizers include diethyl phthalate, tributyl citrate and triacetin.

Composition H (Enteric-coated controlled release tablet)

Enteric-coated tablets of Composition F can be prepared by coating the tablets with 50mg/tablet of an enteric polymer such as cellulose acetate phthalate, polyvinylacetate phthalate, hydroxypropylmethyl- cellulose phthalate, or anionic polymers of methacrylic acid and methacrylic acid methyl ester (Eudgragit L). Except for Eudgragit L, these polymers should also include 10% (by weight of the quantity of polymer used) of a plasticizer to prevent membrane cracking during application or on storage. Suitable plasticizers include diethyl phthalate, tributyl citrate and triacetin.

(ii) Capsule compositions

Composition A

Capsules can be prepared by admixing the ingredients of Composition D above and filling two-part hard gelatin capsules with the resulting mixture. Composition B (infra) may be prepared in a similar manner.

Composition B

		mg/capsule
(a)	Active ingredient	250
(b)	Lactose B.P.	143
(c)	Sodium Starch Glycollate	25
(d)	Magnesium Stearate	2
		420

Composition C

		mg/capsule
(a)	Active ingredient	250
(b)	Macrogol 4000 BP	350
		600

Capsules can be prepared by melting the Macrogol 4000 BP, dispersing the active ingredient in the melt and filling two-part hard gelatin capsules therewith.

Composition D

	mg/capsule
Active ingredient	250
Lecithin	100

Arachis Oil	100
	450

Capsules can be prepared by dispersing the active ingredient in the lecithin and arachis oil and filling soft, elastic gelatin capsules with the dispersion.

Composition E (Controlled release capsule)

		mg/capsule
(a)	Active ingredient	250
(b)	Microcrystalline Cellulose	125
(c)	Lactose BP	125
(d)	Ethyl Cellulose	13
		513

The controlled release capsule formulation can be prepared by extruding mixed ingredients (a) to (c) using an extruder, then spheronising and drying the extrudate. The dried pellets are coated with a release controlling membrane (d) and filled into two-part, hard gelatin capsules.

Composition F (Enteric capsule)

		mg/capsule
(a)	Active ingredient	250
(b)	Microcrystalline Cellulose	125
(c)	Lactose BP	125
(d)	Cellulose Acetate Phthalate	50
(e)	Diethyl Phthalat	5
		555

The enteric capsule composition can be prepared by extruding mixed ingredients (a) to (c) using an extruder, then spheronising and drying the extrudate. The dried pellets are coated

with an enteric membrane (d) containing a plasticizer (e) and filled into two-part, hard gelatin capsules.

Composition G (Enteric-coated controlled release capsule)

Enteric capsules of Composition E can be prepared by coating the controlled-release pellets with 50mg/capsule of an enteric polymer such as cellulose acetate phthalate, polyvinylacetate phthalate, hydroxypropylmethylcellulose phthalate, or anionic polymers of methacrylic acid and methacrylic acid methyl ester (Eudragit L). Except for Eudragit L, these polymers should also include 10% (by weight of the quantity of polymer used) or a plasticizer to prevent membrane cracking during application or on storage. Suitable plasticizers include diethyl phthalate, tributyl citrate and triacetin.

(iii) Intravenous injection composition

Active ingredient	0.200g
Sterile, pyrogen-free phosphate buffer (pH 9.0) to	10 ml

The active ingredient is dissolved in most of the phosphate buffer at 35-40^{o}C, then made up to volume and filtered through a sterile micropore filter into sterile 10 ml glass vials (Type 1) which are sealed with sterile closures and overseals.

(iv) Intramuscular injection composition

Active ingredient	0.20 g
Benzyl Alcohol	0.10 g
Glycofurol 75	1.45 g
Water for Injection q.s. to	3.00 ml

The active ingredient is dissolved in the glycofurol. The benzyl alcohol is then added and dissolved, and water added to 3 ml. The mixture is then filtered through a sterile micropore filter and sealed in sterile 3 ml glass vials (Type 1).

(v) <u>Syrup composition</u>

Active ingredient		0.25g
Sorbitol Solution		1.50g
Glycerol		1.00g
Sodium Benzoate		0.005g
Flavour		0.0125ml
Purified Water	q.s. to	5.0ml

The sodium benzoate is dissolved in a portion of the purified water and the sorbitol solution added. The active ingredient is added and dissolved. The resulting solution is mixed with the glycerol and then made up to the required volume with the purified water.

(vi) <u>Suppository composition</u>

	mg/suppository
Active ingredient	250
Hard Fat, BP (Witepsol H15 - Dynamit NoBel)	<u>1770</u>
	2020

One-fifth of the Witepsol H15 is melted in a steam-jacketed pan at 45^{o}C maximum. The active ingredient is sifted through a 200lm sieve and added to the molten base with mixing, using a Silverson fitted with a cutting head, until a smooth dispersion is achieved. Maintaining the mixture at 45^{o}C, the remaining Witepsol H15 is added to the suspension which is stirred to ensure a homogenous mix. The entire suspension is then passed through a 250lm stainless steel screen and, with continuous stirring, allowed to cool to 40^{o}C. At a

temperature of 38-40^{o}C, 2.02g aliquots of the mixture are filled into suitable plastic moulds and the suppositories allowed to cool to room temperature.

(vii) Pessary composition

	mg/pessary
Active ingredient (63lm)	250
Anhydrous Dextrose	380
Potato Starch	363
Magnesium Stearate	7
	1000

The above ingredients are mixed directly and pessaries prepared by compression of the resulting mixture.

(viii) Transdermal composition

Active ingredient	200mg
Alcohol USP	0.1ml
Hydroxyethyl cellulose	

The active ingredient and alcohol USP are gelled with hydroxyethyl cellulose and packed in a transdermal device with a surface area of 10cm^2.